

CLAIMS

1. A monoclonal antibody that specifically binds to a human VEGF with dissociation constant K_d equal to or lower than 0.2 nM.

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2. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.1 nM.

3. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is 10 equal to or lower than 0.08 nM.

4. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.05 nM.

15 5. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.01 nM.

6. The monoclonal antibody of claim 1, wherein the dissociation constant K_d is equal to or lower than 0.005 nM.

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7. The monoclonal antibody of claim 1, wherein the antibody is in a form of scFv.

8. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab.

25 9. The monoclonal antibody of claim 1, wherein the antibody is in a form of fully assembled antibody.

10. The monoclonal antibody of claim 1, wherein the antibody is in a form of scFv and the dissociation constant K_d is measured at about 4°C, 25°C, 37°C or 42°C.

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11. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab and the dissociation constant K_d is measured at about 4°C, 25°C, 37°C or 42°C.

12. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab and 5 the dissociation constant K_d is measured at about 37°C.

14. A monoclonal antibody that specifically binds to a human VEGF and has V_L comprising the amino acid sequence of

X₁X₂X₃X₄TQX₅PSX₆X₇SX₈X₉X₁₀GX₁₁X₁₂X₁₃X₁₄IX₁₅CX₁₆X₁₇SX₁₈X₁₉IX₂₀X₂₁X₂₂X₂₃X₂₄

10 WYQQX₂₅PGX₂₆APX₂₇X₂₈LX₂₉YX₃₀X₃₁X₃₂X₃₃LX₃₄X₃₅GVX₃₆X₃₇RFSGX₃₈X₃₉SGTDF

X₄₀LTIX₄₁X₄₂LQX₄₃X₄₄DX₄₅AX₄₆YYCQQX₄₇X₄₈X₄₉X₅₀PX₅₁TFGX₅₂GTX₅₃X₅₄IK,

wherein the underlined regions are designated as $V_L/CDR1$, $V_L/CDR2$, and $V_L/CDR3$, respectively, whereas the rest of the region is designated as framework, and wherein X₁ is D, E or A; X₂ is I, or T; X₃ is V, E, K, R, Q, or T; X₄ is M, or L; X₅ is S, or T, X₆ is S, or T; X₇ is L, or V; X₈ is A, or V; X₉ is S, or T; X₁₀ is P, V, L, A, or I; X₁₁ is E, or D; X₁₂ is R, or T; X₁₃ is A, or V I; X₁₄ is T, or A; X₁₅ is T, S, or A; X₁₆ is S, R, N, K, H, or Q; X₁₇ is A, or S; X₁₈ is Q, or R; X₁₉ is S, D, A, or P; X₂₀ is S, G, R, T, or Y; X₂₁ is T, N, S, D, or K; X₂₂ is Y, or D; X₂₃ is L, or I; X₂₄ is A, N, or T; X₂₅ is K, or I; X₂₆ is Q, K, T, or I; X₂₇ is R, K, Q, N, H, S, or E; X₂₈ is V, or L; X₂₉ is I, or V; X₃₀ is F, A, G, D, or S; 20 X₃₁ is A, or T; X₃₂ is S, or T; X₃₃ is N, S, R, or T; X₃₄ is A, H, or Q; X₃₅ is S, or G; X₃₆ is P, T; X₃₇ is S, N, D, G, or Y; X₃₈ is S, or T; X₃₉ is G, or R; X₄₀ is T, or A; X₄₁ is S, or R; X₄₂ is S, or R; X₄₃ is P, or A; X₄₄ is E, or D; X₄₅ is F, V, or S; X₄₆ is V, T, I, A, or S; X₄₇ is Y, or S; X₄₈ is S, Y, or N; X₄₉ is S, or T; X₅₀ is T, V, A, P, K, G, S, or I; X₅₁ is W, or Y; X₅₂ is Q, or G; X₅₃ is V, or L; and X₅₄ is E, D, or A.

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15. A monoclonal antibody that specifically binds to a human VEGF and has V_L comprising the amino acid sequence of

X₁X₂X₃LTQPPSX₄SX₅TPGQX₆VTISCSGX₇X₈SNX₉GX₁₀NX₁₁VX₁₂WYQQX₁₃PGX₁₄A

PKX₁₅LX₁₆YX₁₇NX₁₈X₁₉RPSGVPX₂₀RX₂₁SGSX₂₂SX₂₃TSASLAISGLX₂₄SEDEADYY

30 CX₂₅X₂₆WDDDSLX₂₇GYVFGX₂₈GTX₂₉LTVL, wherein the underlined regions are designated as $V_L/CDR1$, $V_L/CDR2$, and $V_L/CDR3$, respectively, whereas the rest of the

region is designated as framework, and wherein X₁ is Q L, or N; X₂ is P A F, or S; X₃ is V, or M; X₄ is A, or T; X₅ is G, or A; X₆ is R, or S; X₇ is S, or T; X₈ is S, T Y, or N; X₉ is I, or V; X₁₀ is S, or R; X₁₁ is S, P, N, A, or T; X₁₂ is N, T , or Y; X₁₃ is L, or F; X₁₄ is T, or A; X₁₅ is V, L, or F; X₁₆ is M, or I; X₁₇ is G, T, or S; X₁₈ is N, or D; X₁₉ is Q, or E; X₂₀ is D, or E; X₂₁ is F, or L; X₂₂ is K, or R; X₂₃ is G, or A; X₂₄ is Q, L, or R; X₂₅ is A, or G; X₂₆ is A, S, or T; X₂₇ is N, S, or T; X₂₈ is T, or A; and X₂₉ is K, or Q.

16. A monoclonal antibody that specifically binds to a human VEGF and has V_L comprising the amino acid sequence of
- 10 QSALTQPPSVSGAPGQRVTISCTGRSSNIGAGHDVHWYQQLPGTAPKLLIYANDQ RPSGVPDFSDSKSGTSASLGISGLRSEDEADYFCATWDDSLHGYVFGTGTKVTV L (SEQ ID No: 54).
17. A monoclonal antibody is provided that specifically binds to a human VEGF and has V_H comprising the amino acid sequence of
- X₁X₂QLVX₃SGGGX₄VQPGGX₅LRLX₆CAX₇SGX₈X₉X₁₀X₁₁X₁₂X₁₃GX₁₄NWX₁₅RQAP GKGX₁₆EWVGWX₁₇NTX₁₈X₁₉GX₂₀X₂₁TYX₂₂X₂₃X₂₄FX₂₅RRX₂₆TX₂₇SX₂₈X₂₉X₃₀SKX₃₁X₃₂X₃₃YLQX₃₄NSLRAEDTAVYYCAX₃₅YPX₃₆YYGX₃₇SHWYFDVWX₃₈QGTLVTV SS, wherein the underlined regions are designated as CDR1, CDR2, and CDR3,
- 20 respectively, whereas the rest of the region is designated as framework according to Kabat nomenclature, and wherein X₁ is E, or Q; X₂ is V, or G; X₃ is Q, or E; X₄ is V, or L; X₅ is S, or T; X₆ is S T, or R; X₇ is A, or V; X₈ is Y, or F; X₉ is T, D, N, S, or A; X₁₀ is F, or L; X₁₁ is T, D, Y, A, S, or N; X₁₂ is N, H, or S; X₁₃ is Y, or F; X₁₄ is M, L, I, or V; X₁₅ is I, V, or L; X₁₆ is L, or P; X₁₇ is I, or V; X₁₈ is Y, or N; X₁₉ is T, or N; X₂₀ is E, or A; X₂₁ is P, T, or S; X₂₂ is A, or V; X₂₃ is A, H, Q, P, D, or E; X₂₄ is D, or E; X₂₅ is K, or T; X₂₆ is V, F, or L; X₂₇ is F, or I; X₂₈ is L, or R; X₂₉ is D, or N; X₃₀ is T, or N; X₃₁ is S, or N; X₃₂ is T, Q, P, or K; X₃₃ is A, V, or P; X₃₄ is L, or M; X₃₅ is K, or R; X₃₆ is H, or Y; X₃₇ is S, R, or T; and X₃₈ is G, or A.
- 30 18. A monoclonal antibody is provided that specifically binds to a human VEGF and has V_L comprising the amino acid sequence selected from the group consisting of SEQ ID

- NOs:2-54, more preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:47, and SEQ ID NO:54.
- 5 19. A monoclonal antibody that specifically binds to a human VEGF and has V_H comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:57-110 and SEQ ID NOs:285-310, and preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:61-64, SEQ ID NO:67, 68, 70, 75, 83, 88, 89, 90, 91, 92, 93, 94, and 96-110.
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- 20. A monoclonal antibody is provided that specifically binds to a human VEGF and has CDR2 in the V_L region ($V_L/CDR2$) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:195-209.
- 15 21. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the V_L region ($V_L/CDR3$) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:210-228.
- 22. A monoclonal antibody that specifically binds to a human VEGF and has a framework region (FR) CDR3 in the V_L region (V_L/FR) comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:229-269, and preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NO:232, 235, 237, 251, 255, 263, and 265.
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- 25 23. A monoclonal antibody that specifically binds to a human VEGF and has CDR1 in the V_H region ($V_H/CDR1$) comprising the amino acid sequence of $GX_1X_2X_3X_4X_5X_6GX_7N$, wherein X_1 is Y, or F; X_2 is D, N, T, S, or A; X_3 is F, or L; X_4 is T, D, S, Y, A, or N; X_5 is H, N, or S; X_6 is Y, or F; X_7 is M, L, I, or V.
- 30 24. A monoclonal antibody that specifically binds to a human VEGF and has CDR2 in the V_H region ($V_H/CDR2$) comprising the amino acid sequence of

WX₁NTX₂X₃GEX₄TYX₅X₆X₇FX₈R, wherein X₁ is I, or V; X₂ is Y, or N; X₃ is T, or N; X₄ is P, T, or S; X₅ is A, or V; X₆ is A, Q, P, H, D, or E; X₇ is D, or E; and X₈ is K, or T.

25. A monoclonal antibody that specifically binds to a human VEGF and has CDR2 in the V_H region (V_H/CDR2) comprising the amino acid sequence selected from the group consisting of: SEQ ID NOs:136-156.
26. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the V_H region (V_H/CDR3) comprising the amino acid sequence of KYPX₁YYGX₂SHWYFDV, wherein X₁ is Y, or H, and X₂ is R.
27. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the V_H region (V_H/CDR3) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:311-337.
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28. A monoclonal antibody that specifically binds to a human VEGF and has FR in the V_H region (V_H/FR) comprising the amino acid sequence of X₁VQLVX₂SGGGX₃VQPGGX₄LRLX₅CAX₆S/CDR1/WX₇RQAPGKGLEWVG/CDR2//RX₈TX₉SX₁₀DX₁₁SKX₁₂X₁₃X₁₄YLQX₁₅NSLRAEDTAVYYCA/CDR3/WX₁₆QGTLVTV
- 20 SS, wherein X₁ is E, or Q; X₂ is Q, or E; X₃ is V, or L; X₄ is S, or T; X₅ is S, T, or R; X₆ is A, or V; X₇ is I, or V; X₈ is F, or V; X₉ is F, or I; X₁₀ is L, or R is X₁₁ is T, or N; X₁₂ is S, or N; X₁₃ is T, Q, or K; X₁₄ is A, or V; X₁₅ is M, or L; and X₁₆ is G, or A.
29. A monoclonal antibody that specifically binds to a human VEGF and has a V_L and V_H pair selected from the group consisting of: SEQ ID NO:1 and 70; SEQ ID NO:1 and 67; SEQ ID NO:1 and 75; SEQ ID NO:1 and 83; SEQ ID NO:14 and 55; SEQ ID NO:1 and 101; SEQ ID NO:1 and 100; SEQ ID NO:14 and 102; SEQ ID NO:1 and 103; SEQ ID NO:1 and 104; SEQ ID NO:1 and 105; SEQ ID NO:36 and 100; SEQ ID NO:26 and 100; SEQ ID NO:28 and 100; SEQ ID NO:37 and 100; SEQ ID NO:44 and 100;
- 30 SEQ ID NO:54 and 100; and SEQ ID NO:47 and 100, preferably selected from the group consisting of SEQ ID NO:28 and 61; SEQ ID NO:28 and 62; SEQ ID NO:28 and 63;

SEQ ID NO:28 and 64; SEQ ID NO:28 and 68; SEQ ID NO:28 and 85; SEQ ID NO:28 and 86; SEQ ID NO:28 and 87; SEQ ID NO:28 and 88; SEQ ID NO:28 and 89; SEQ ID NO:28 and 90; SEQ ID NO:28 and 91; SEQ ID NO:28 and 92; SEQ ID NO:28 and 93; SEQ ID NO:28 and 94; SEQ ID NO:28 and 95; SEQ ID NO:28 and 96; SEQ ID NO:28 and 97; SEQ ID NO:28 and 98; SEQ ID NO:28 and 99; SEQ ID NO:28 and 106; SEQ ID NO:28 and 107; SEQ ID NO:28 and 108; SEQ ID NO:28 and 109; and SEQ ID NO:28 and 110.

5 30. The monoclonal antibody of any of claims 14-29, wherein the antibody has
10 dissociation constant K_d equal to or lower than 10 nM.

31. The monoclonal antibody of any of claims 14-29, wherein the antibody has
dissociation constant K_d equal to or lower than 1 nM.

15 32. The monoclonal antibody of any of claims 14-29, wherein the antibody has
dissociation constant K_d equal to or lower than 0.1 nM.

33. The monoclonal antibody of any of claims 14-29, wherein the antibody has
dissociation constant K_d equal to or lower than 0.01 nM.